to activating Ca^{2+} in a site-dependent manner. The calculated CICR activity strongly correlated with the ER Ca^{2+} level, an index of Ca^{2+} leak. Importantly, the accelerated sensitivity to activating Ca^{2+} was linked to pathogenesis of CCD. Overall, the effects were similar to those of the amino-terminal mutations. The underlying molecular mechanism will be discussed.

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Characterization of Dual Mutant RyR1D-S100A1KO Mice with Disrupted CaM and S100A1 Binding to CaMBD2 and Lacking S100A1 Expression

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S100A1 and calmodulin (CaM) modulate skeletal muscle Ca^{2+} signaling. Recently, isothermal titration calorimetry demonstrated that CaM binds to three regions within the ryanodine receptor type-1 (RyR1) sequence: CAMBD1, residues 1975-1999; CaMBD2, residues 3614-3640; CAMBD3, residues 4295-4325 (Lau K, et al., 2014). We previously studied the binding to and modulation of RyR1 by CaM and S100A1 via CaMBD2, showing that skeletal muscle fibers from S100A1 knock out (S100A1KO) mice or RyR1-L3625D (RyR1D) mice (having disrupted CaM and S100A1 binding to CaMBD2), exhibit impaired RyR1 regulation. Here we propose and test the hypothesis that S100A1 also modulates fiber activation via sites other than CaMBD2. Double mutant mice were generated by crossbreeding homozygous RyR1D and S100A1KO mice to obtain homozygous double mutant RyR1D-S100A1KO mice, which were viable and appeared normal. Homozygous RyR1D mice were used as controls to eliminate any effect of binding at CaMBD2. In vivo stimulation of tibialis anterior muscles in anesthetized mice showed that maximal specific force was reduced in RyR1D-S100A1KO compared with RyR1D muscles (RyR1D Po= 5.15 ± .09 g/mg, RyR1D-S100A1KO Po= 4.05 ± .09 g/mg; $p < 0.05$). Using high-speed confocal microscopy of Ca^{2+} indicator rhod-2, we show that RyR1D-S100A1KO flexor digitorum brevis muscle fibers exhibit increased global myoplasmic Ca^{2+} transients following field stimulation. We also found no differences in myoplasmic resting Ca^{2+} between RyR1D and RyR1D-S100A1KO. Our results indicate that RyR1D-S100A1KO muscles have increased SR Ca^{2+} release, however exhibit reduced force generation. These results suggest that S100A1, similar to CaM, binds to different regions within the RyR1, but also indicates that S100A1 could bind to other target proteins (i.e., contractile apparatus). Where and how exactly S100A1 exerts these additional effects is currently unknown. Support: NIH R37-AR055099 and R01-AR059179.

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Correlation between FKBP12/12.6 Bound to RyRs, Channel Opening to Maximal or Submaximal Conductance Levels and Myopathy

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Ryanodine receptor (RyR) Ca^{2+} release channels located in the membrane of sarcoplasmic reticulum Ca^{2+} stores provide the Ca^{2+} required for contraction in skeletal muscle and the heart. The normal function of the ion channels is necessary for optimal muscle function. Increases in channel activity in relaxed muscle lead to increased cytoplasmic Ca^{2+}, skeletal myopathy and cardiac arrhythmia. The 12.0 and 12.6 KDa proteins that bind to the immunosuppressant drug FK506, FKBP12 and FKBP12.6, also bind to RyR channels and the binding is thought to be essential for normal channel function. Their dissociation from the RyR has been associated with the damaging increase in ''leak'' of Ca^{2+} from the SR when the muscle is relaxed. However the extent to which dissociation of the FKBPs from RyRs contributes to skeletal and cardiac myopathies caused by ''leaky'' RyR channels is unclear. In addition, there is controversy about the changes in RyR channel gating, in particular the prevalence of submaximal conductance opening, that contribute to channel ''leak''. We have addressed this problem by examining association of the FKBP12 and FKBP12.6 in two myopathic conditions. Firstly in the presence of a mutant CLIC-2 protein, that activates RyR2 and whose interaction with RyR2 has been associated with cardiac hyperthrophy and intellectual deficit [1]. Secondly, with a cholesterol-reducing drug simvastatin that can produce a skeletal myopathy. In both cases we show increases in channel activity, particularly in the number and duration of submaximal conductance openings, as well significant FKBP12 and 12.6 dissociation from RyR2 and FKBP12 dissociation from RyR1. The results suggest that, in these situations, ''leak'' through RyR channels is facilitated by increased sub-conductance opening, due to dissociation or destabilisation of FKBP binding.Takano et al., Hum Mol Genet. 2012;21(20):4497-507.

Cardiac Smooth and Skeletal Muscle Electrophysiology II

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A Comparison of Acutely Isolated Human Ventricular Myocytes with Stem Cell Derived Cardiocytes

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Recent studies have characterized cardiac ionic currents in cardiocytes derived from human induced pluripotent stem cells. However, direct comparison with human ventricular myocytes is hampered by differences in experimental conditions. Similarly, stem cell derived cardiocytes are different and potentially more variable than native myocytes. We examined commercially prepared cells (iCells) and scale production cells (hIPS-CMs) using electronic expression of IK1 to distinguish ventricular cells, compared with myocytes (VM) from biopsy samples.

APs were recorded in VM, iCells and hiPSC-CMs. iCells and hiPSC-CMs had depolarized membrane potentials, slower upstroke velocities, and prolonged AP relative to VM. These differences were minimized by electronic expression of IK1. Capacitances were larger in VM (298.97 ± 27.5 pF (4 hearts, n=44)) than in iCells (73.21 ± 5.4 (n=32), $p<0.05$) and hIPS-CMs (64.25 ± 4.94 (n=75) ,$p<0.05$). At −120 mV, a large IK1 was observed in VM (−14.3 ± 1.36 pA/pF (4 hearts, n=44)); IK1 was much smaller in iCells (−1.73 ± 0.92 (n=19), $p<0.01$) and hIPS-CMs (−2.54 ± 0.49 (n=29), $p<0.01$). VM had larger peak outward (Ito) currents at +50 mV (6.2 ± 0.7 pA/pF (n=44, four hearts)) than iCells (2.6 ± 0.2 (n=19, $p<0.01$)) and hIPS-CMs (2.9 ± 0.3 (n=29, $p<0.01$)) but all had similar variability relative to current density. Large sodium currents were recorded at 0 mV in VM (−23.7 +/− 3.4 pA/pF (4 hearts, 32 myocytes)) as well as in iCells (−26.1 +/− 3.2 (n=14)) and in hiPSC-CMs (−17.0 +/− 4.1 (n=16)) with high degrees of variability for all cell types. In conclusion, there are functional differences between VM and stem cell derived cell types, but minimal differences between stem cell derived cardiocytes based on source. Similarly, cell to cell variability was similar regardless of source.

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Aging Alters cAMP Signaling and Membrane-Delimited Regulation of if in Sinoatrial Myocytes

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We recently demonstrated that age-dependent declines in intrinsic and maximum heart rates are associated with slower action potential (AP) firing rate and a hyperpolarizing shift in the midpoint activation voltage ($V_{1/2}$) of the funny current (I_f) in isolated sinoatrial myocytes (SAMs). Given that cAMP is an important intracellular modulator in SAMs, we hypothesized that reduced cAMP concentration may contribute to the slower APs and shifted I_f in aged SAMs. To test this hypothesis, we applied a saturating concentration of cAMP via the patch pipette in whole cell voltage-clamp and current clamp experiments in acutely isolated SAMs from young and old mice. Here, we show that this exogenous cAMP completely reversed the age-dependent changes in both AP firing rate and I_f in SAMs. In contrast, maximal stimulation of endogenous cAMP production, via co-application of the PDE inhibitor, IBMX and the adenylyl cyclase activator, forskolin, did not rescue either AP firing rate or I_f in aged SAMs. These data indicate that a deficit in cAMP production and/or responsiveness may contribute to the age-dependent changes in SAM excitability. To further determine how cAMP regulates I_f in aged versus young SAMs, we compared I_f activation in excised inside-out membrane patches from SAMs from young and old mice. Surprisingly, we found that the age-dependent hyperpolarized shift in the $V_{1/2}$ of I_f persisted in the cell-free patches, indicating that soluble cAMP alone cannot account for the shifted I_f in aged SAMs. Rather, aging appears to alter an additional, membrane-associated factor that regulates I_f in SAMs. Future work will focus on resolving the underlying mechanism(s) responsible for the age-dependent shift in the $V_{1/2}$ of I_f, and its relationship to AP generation in SAMs.